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(54) Title: STABILIZED AQUEOUS PEPTIDE SOLUTIONS (57) Abstract Aqueous compositions comprising at least one peptide selected from glucagon, GLP-1, and analogues and derivatives thereof together with a stabilizing and solubilizing amount of at least one detergent, said detergent having at least 2 positive charges, at least 2 negative charges, or a combination of at least one positive charge and at least one negative charge.		

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STABILIZED AQUEOUS PEPTIDE SOLUTIONS

FIELD OF THE INVENTION

- 5 The present invention relates to stable, injectable aqueous solutions of peptides such as glucagon, glucagon-like peptide-1 (GLP-1, e.g. GLP-1(7-37) and GLP-1(7-36) amide) and analogues and derivatives thereof.

BACKGROUND OF THE INVENTION

10

Glucagon and glucagon-like peptide 1 (GLP-1) are polypeptide hormones both derived from proglucagon. Upon tissue-specific processing, glucagon is produced in the pancreas, while GLP-1 is predominantly secreted in the intestine. Physiologically, both peptides play major roles in the regulation of blood glucose. Glucagon is directly
15 involved via glycogenolytic as well as gluconeogenic effects. GLP-1 is indirectly involved via stimulation of insulin and inhibition of glucagon secretion. Thus, the two peptides affect the blood glucose in opposite directions.

Due to its glycogenolytic effect on the liver, glucagon is used for the treatment of
20 acute hypoglycemia (e.g. "insulin shock"). In addition, glucagon exerts a spasmolytic effect on smooth muscles, and this effect is used clinically in connection with several imaging procedures, especially radiology.

GLP-1 fragments such as GLP-1(7-37), GLP-1(7-36) amide and functional analogues
25 thereof (hereinafter designated GLP-1 compounds) stimulate pancreatic insulin release in the presence of glucose, and this finding suggests that GLP-1 compounds may be useful in the treatment of type II diabetes.

Glucagon (29 amino acids; see e.g. *The Merck Index*, 10th Edition for the amino acid
30 sequence) and GLP-1 compounds (typically about 29-31 amino acids) are single chain highly homologous peptides. While little information is available on the solution behaviour of GLP-1 compounds, the physico-chemical properties of glucagon are well described in the literature (*J.Biol.Chem.* 244, 6675-6679 (1969); *Nature* 257, 751-757 (1975); *FEBS Letts.* 119, 265-270 (1980)). The peptide exhibits very low

solubility in water at a pH in the range of about 4-9. Outside this region, aqueous solutions are generally metastable and prone to gel formation or flocculation within hours of their preparation.

- 5 Accordingly, glucagon compositions for injection are currently marketed in the form of a lyophilized powder which is to be reconstituted using a suitable diluent shortly before use. The resulting acidic solution may then be injected. In contrast with the single-dose compositions of glucagon used for acute hypoglycemia and the motility indication in radiology, the utility of GLP-1 compounds for the treatment of type II
10 diabetes generally involves multiple-dose soluble compositions. Given the instability of glucagon, the formulation of such compositions is inherently difficult.

Thus, there is a need for stable aqueous formulations of peptides such as glucagon and GLP-1 compounds. In the case of glucagon, the availability of a ready-to-use
15 composition is desirable for emergency treatment of acute hypoglycemia, enabling the patient or another person present to treat the hypoglycemic incidence immediately. Also for the mobility indication for glucagon used in radiology, and for the type II diabetes indication for GLP-1 compounds, the availability of a ready-to-use aqueous composition provides obvious advantages in terms of convenience and safety.

20

Glucagon compositions solubilized by the addition of detergent have received relatively little attention in the literature. British patent No. 1 202 607 describes the use of either cationic (quarternary ammonium bases) or anionic (alkyl-aryl-sulphonates), monovalent detergents. These compounds provide enhanced solubilization of 1 mg/ml
25 glucagon over a limited pH range using a 6-fold molar excess of detergent.

A series of other studies on glucagon-detergent interactions employ much lower glucagon concentrations and do not address the effect of detergent on peptide solubility. Thus, in studies using 0-200 μ M of the amphoteric (twitterionic) detergent
30 hen egg lysolecithin, binding of detergent was shown to induce partial helical structure in solutions of glucagon containing about 0.02 mg/ml peptide (J.Biol.Chem. 247, 4986-4991; 4992-4995; (1972)). Similarly, in studies employing dilute (0.005-0.05 mg/ml) solutions of glucagon, lysolecithin was found more efficient than the anionic detergents dipalmitoyl phosphatidic acid and sodium dodecyl sulfate (SDS) in inducing

partial helical structure in glucagon (Biopolymers 21, 1217-1228 (1982); Biopolymers 22, 1003-1021 (1983)).

At the other extreme, a few examples are available characterizing the interaction
5 between glucagon and concentrated detergent solutions. These include the binding of
dilute glucagon to micelles of the anionic detergent SDS at 25 mM, pH 2
(Biochemistry 19, 2117-2122, (1980)), and the solubilization of glucagon (up to 20
mg/ml) via binding to micelles of the amphoteric dodecylphosphocholine present in a
40-fold molar excess (e.g. 3.5 mg/ml glucagon is solubilized using 40 mM detergent
10 at neutral pH; Biochim.Biophys.Acta 603, 298-312, (1980))

With the exception of a single example describing the solubilization of 20 mg/ml
GLP-1(7-36) amide bound to micelles of dodecylphosphocholine at 280 mM (cf. the
glucagon case above, Biochemistry 33, 3532-3539, 1994), no reports are available on
15 the binding of detergents by GLP-1 compounds and the resulting effect on peptide
solubility and stability. In contrast, several ways of preparing prolonged delivery of
GLP-1 compounds have been described (e.g. European patent application No.
0619322 and column 6 of US patent No. 5,120,712). The possibilities include use of
polymers, incorporation into particles of polymer material or into an oil suspension,
20 addition of zinc ion, protamine sulphate and/or phenolic compounds, and preparation
of amorphous/crystalline formulations.

BRIEF DESCRIPTION OF THE INVENTION

25 It has now surprisingly been found that soluble and stable formulations of glucagon
and GLP-1 compounds may be prepared by means of addition of detergents carrying
multiple charges (i.e. two or more negative, two or more positive, or both positive and
negative charges). When added in an amount corresponding to e.g. about 0.5-20
moles of detergent per mole of peptide, this class of detergents is capable of
30 solubilizing pharmaceutically relevant concentrations of glucagon and GLP-1
compounds in the entire pH range from 4 to 9. The expanded pH range allows optimal
selection of conditions to suppress chemical degradation of the solubilized peptide.
Furthermore, preferred detergents consist of naturally occurring building blocks which
are more biologically acceptable than those previously described.

Thus, in its broadest aspect, the present invention relates to a pharmaceutical composition in the form of a solution comprising a polypeptide containing an amphiphatic α -helix which is stabilized by interaction with a detergent carrying
5 multiple charges. Such polypeptides include, but are not limited to, glucagon, GLP-1 compounds, corticotropin (ACTH), calcitonin, and functional analogues thereof.

One aspect of the invention relates to an aqueous composition in the form of a solution comprising at least one peptide selected from glucagon, GLP-1, and
10 analogues and derivatives thereof together with a stabilizing and solubilizing amount of at least one detergent, said detergent having at least 2 positive charges, at least 2 negative charges, or a combination of at least one positive charge and at least one negative charge, the peptide or analogue or derivative thereof being present in a concentration of at least about 0.1 mg/ml, with the proviso that the detergent is not
15 dodecyl phosphocholine.

Another aspect of the invention relates to a method for stabilizing an aqueous composition in the form of a solution comprising at least one peptide selected from glucagon, GLP-1 and analogues and derivatives thereof by adding to the composition a
20 stabilizing and solubilizing amount of at least one such detergent having multiple charges.

In a further embodiment, the invention relates to a method for treating insulin dependent or non-insulin dependent diabetes mellitus or obesity in a patient in need of such
25 treatment, comprising administering to the patient a therapeutically effective amount of an appropriate stabilized aqueous composition as described herein.

DETAILED DESCRIPTION OF THE INVENTION

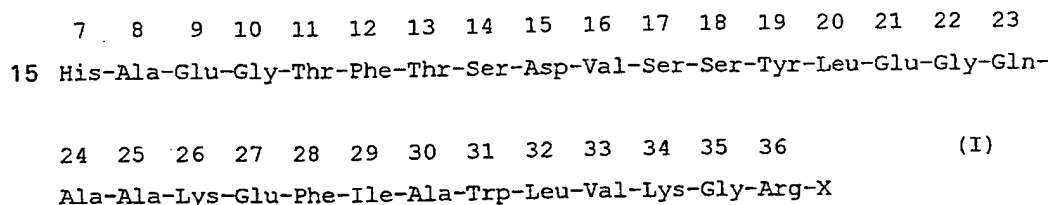
30 Glucagon and GLP-1 compounds belong to a class of polypeptides which have the potential of forming amphiphatic helices, i.e., one face of the helical structure displays mainly hydrophobic amino acid side chains, while the other face exposes hydrophilic and/or charged side chains. In polypeptides of this type, the formation of the

amphiphatic helical structure is often linked to inter-peptide helix self-association along the interfaces defined by the hydrophobic patches.

A simple system is used to describe fragments and analogues of GLP-1. Thus, for example, Gly⁸-GLP-1(7-37) designates a fragment of GLP-1 formally derived from GLP-1 by deleting the amino acid residues Nos. 1 to 6 and substituting the naturally occurring amino acid residue in position 8 (Ala) by Gly. Similarly, Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-37) designates GLP-1(7-37) wherein the ε-amino group of the Lys residue in position 34 has been tetradecanoylated.

10

The amino acid sequence of GLP-1(7-36)amide and GLP-1(7-37) is as given in the general formula (I) below:



20 which shows GLP-1(7-36)amide when X is NH₂ and GLP-1(7-37) when X is Gly-OH.

In the present text, the designation "analogue" is used to designate a peptide wherein one or more amino acid residues of the basic GLP-1 peptide have been substituted by another amino acid residue and/or wherein one or more amino acid residues of the peptide have been deleted and/or wherein one or more amino acid residues have been added to the peptide. Such addition can take place either at the N-terminal end or at the C-terminal end or both. The term "derivative" is used in the present text to designate a peptide in which one or more of the amino acid residues of the peptide have been chemically modified, e.g. by alkylation, acylation, ester formation or amide formation. For the sake of simplicity, the term "peptide" will generally be used herein to refer to the glucagon and GLP-1 peptides as such as well as analogues and derivatives thereof.

The present invention is based on situations where the hydrophilic patch of the putative amphiphatic helix displays two or more charges. By the addition of a

detergent carrying a complementary set of multiple charges, a stabilizing and solubilizing helix-forming effect will result from a combination of two factors, i.e. 1) charge-charge interactions between the polar head of the detergent and the charged amino acid side chains of the helix; and 2) the hydrophobic tail of the detergent
5 serving as a template for association with the hydrophobic patch of the same or that of a neighboring helix.

The stabilized solutions of the invention can be used for parenteral, pulmonal or nasal administration. Such solutions remain stable and retain biological activity when stored
10 at temperatures of e.g. 4°-25°C for extended periods of time.

Suitable detergents according to the invention are characterized by having multiple charges, i.e. two or more positive charges, two or more negative charges, or a combination of at least one positive charge and at least one negative charge. The
15 advantage of multiple charges as opposed to monovalent ionic detergents is illustrated in the appended Figure 1. This figure compares the pH-dependence of the equilibrium solubility in 1 mg/ml solutions of glucagon in the absence of additives and in the presence of 1 mM of each of the following detergents: a) cetyl-trimethylammonium bromide (CTAB, i.e. monovalent cationic), b) sodium dodecyl sulfate (SDS, i.e.
20 monovalent anionic) and c) myristoyl lysophosphatidylcholine (LPCM, i.e. amphoteric).

These data show that, while the monovalent cationic and anionic detergents promote solubility at the acidic and basic limbs of the solubility curve, respectively, the amphoteric detergent effects full solubility over the entire pH range. Furthermore, the
25 effect of the amphoteric detergent is clearly much stronger than that expected from the sum of the effects seen with each of the monovalent detergents.

The detergents used in accordance with the present invention contain a polar head group with two or more charges and one or more aliphatic side chains each having at
30 least 6 carbon atoms. In the preferred detergents, any aliphatic side chains have 8-20, e.g. 10-18 carbon atoms. With respect to the polar head group, useful detergents include those selected from the group consisting of lysophospholipids (e.g. 1-acyl-sn-glycero-3-phosphate esters of ethanolamine, choline, serine or threonine). Lauroyl and myristoyl derivatives of lysophosphatidylcholine, lysophosphatidylserine and

lysophosphatidylthreonine are particularly useful. Alternatively, the polar group may be a basic amino acid (e.g. N^α-acylated derivatives of lysine, arginine or histidine, or side-chain acylated derivatives of lysine or arginine). It may also be a dipeptide (e.g. N^α-acylated derivatives of dipeptides comprising any combination of lysine, arginine or
5 histidine and a neutral or acidic amino acid) or a tripeptide (e.g. an N^α-acylated derivative of a tripeptide comprising any combination of a neutral amino acid and two charged amino acids). The polar head group may also be selected from the group of betaines, i.e. amphoteric compounds where the basic group is quarternary ammonium, sulfonium or phosphonium and the acidic group is carboxylate, carboxyalkyl, sulfonate
10 or propanesulfonate. Finally, the detergent may be selected from the group of imidazoline derivatives. It is preferred, however, that the detergent is not dodecyl phosphocholine.

An example of a suitable composition according to the invention is one comprising
15 about 0.5-2.5 mg of peptide per ml, e.g. about 1 mg of peptide per ml, and about 1-3 mM, e.g. about 2 mM of the detergent, e.g. lauroyl or myristoyl lysophosphatidylcholine.

The stable aqueous, injectable solutions of glucagon and GLP-1 compounds according
20 to the invention may be prepared by bringing together the peptide, water and a stabilizing and solubilizing amount of detergent. Typically, the solutions will contain other excipients to adjust ionic strength and provide buffering capacity and antibacterial action as needed. The final peptide concentration will be at least about 0.1 mg/ml, typically about 0.2-5 mg/ml, e.g. about 0.5-3 mg/ml, and the detergent is
25 typically employed in an amount corresponding to at least about 0.3 mole of detergent per mole of peptide, more typically at least about 0.5 mole detergent/mole of peptide and up to about 40 moles detergent/mole of peptide, typically up to about 30 moles detergent/mole peptide, e.g. about 1-20 moles detergent/mole peptide, such as about 1-10 moles detergent/mole peptide. For a given peptide and detergent and a given
30 concentration of peptide, a person skilled in the art will readily be able to determine a suitable detergent concentration that results in the desired stabilization. Isotonic ionic strength may be provided by conventional pharmaceutically acceptable agents such as NaCl, glycerol or mannitol. pH is buffered in the desired range between about 4 and about 8, typically by using acetate, citrate or phosphate buffers at 1-10 mM

concentration as is well-known in the art. Finally, when a preservative is needed, appropriate antibacterial action is secured by the addition of, typically, phenol, m-cresol or benzyl alcohol or mixtures thereof.

- 5 The compositions of the invention can be used in the treatment of various diseases that can be treated by administering glucagon, GLP-1, or analogues or derivatives thereof. The particular peptide to be used and the optimal dose level for any patient will depend on the disease to be treated and on a variety of factors, including the efficacy of the specific peptide employed, the age, body weight, physical activity, and diet of the
- 10 patient, on a possible combination with other drugs, and on the severity of the case. A typical dose of GLP-1 for regulation of the blood sugar level may e.g. be about 0.5-1 mg, for example once a day. A typical dose for glucagon may be approximately 1 mg, e.g. for gastro-intestinal imaging. It is recommended that the dosage of the active compound in question be determined for each individual patient in each case by
- 15 those skilled in the art.

The invention will be further illustrated by the following non-limiting examples.

EXAMPLES

20

Materials & Methods

EQUILIBRIUM SOLUBILITY

- For pH-solubility profiles, solutions containing the peptide and additives (detergent
- 25 and, where indicated, other excipients) at the appropriate concentrations were prepared at pH 9-10. The solutions were filtered, samples were withdrawn, and the pH was adjusted to the desired value in the range of 3-8. The samples were left for 24 hours at 23°C to attain solubility equilibrium. After centrifugation (20,000 g for 20 minutes, 23°C) of each sample, the pH was measured, and the solubility was
- 30 determined from measurement of the absorbance at 276 nm of the supernatant.

Long term physical stability

Glucagon and GLP-1(7-37) were dissolved at twice the desired final concentration and incubated briefly (<10 minutes) at pH 11.5, 23°C before filtration and mixing with an equal volume of a filtered solution containing all the excipients in twice the desired final concentration. The pH was then measured and adjusted as needed. The solution was transferred to pen-fill cartridges containing a small glass ball (to allow visual determination of changes in solution viscosity). The containers were sealed and incubated at the desired temperature between 4°C and 37°C. At appropriate time intervals, the samples were gently turned and visually examined using a light box.

10 When physical changes were apparent (precipitation, crystallization or gelation), the sample was centrifuged and the absorbance was measured in the supernatant to determine whether the component coming out of solution was the peptide or not.

Example 1

15

Equilibrium solubility was determined as a function of pH for 1 mg/ml glucagon solutions in the absence of additives and in the presence of 1 or 2 mM of the detergent LPCL (lauroyl lysophosphatidylcholine). The results are shown in Figure 2. It may be seen that excellent results, i.e. approximately 100% solubility throughout the pH range tested, were obtained with both concentrations of LPCL. For the control solution without additives, acceptable solubility was only obtained at pH 3.

20

Example 2

25 Equilibrium solubility was determined as a function of pH in the pH range of 6-8 for 2 mg/ml solutions of glucagon in the absence of additives and in the presence of 2.5 mM of the detergents LPSP (palmitoyl lysophosphatidyl-L-serine) and LPCM (myristoyl lysophosphatidyl choline). The results are shown in Figure 3. Both additives provided 100% solubility within the tested pH range of 6-8.

30

Example 3

Equilibrium solubility was determined as a function of pH for 1 mg/ml solutions of glucagon in the absence of additives and in the presence of increasing amounts of

LPCM (myristoyl lysophosphatidyl choline). The results are shown in Figure 4. For solutions containing 1 or 2 mM LPCM, approximately 100% solubility was obtained throughout the entire pH range, while 0.5 mM LPCM provided insufficient solubility at pH 4-8. However, even the addition of 0.5 mM LPCM gave a substantial improvement
5 over the control solution.

Example 4

The following examples illustrate various glucagon solutions prepared according to the
10 invention. All of the indicated solutions remained clear, i.e. the glucagon remained completely soluble, for at least the duration of the observation period of four weeks:

mg/ml Glucagon	mM detergent	buffer, pH	Other additive	Temp- erature
2	5.0 LPCM	5 mM phosphate, 7.2		4 °C
2	5.0 LPCM	5 mM phosphate, 7.2	0.2 M mannitol	4 °C
1	5.0 LPCM	5 mM phosphate, 6.0	0.12 M NaCl	4 °C
1	5.0 LPCM	5 mM phosphate, 6.5	0.2 M mannitol	4 °C
1	2.0 LPCM	5 mM phosphate, 7.2		4 °C
1	2.0 LPCM	5 mM phosphate, 7.2	0.12 M NaCl	4 °C
1	2.0 LPCM	5 mM phosphate, 6.0	0.12 M NaCl	4 °C
0.5	1.0 LPCM	5 mM phosphate, 7.2	0.12 M NaCl	4 °C
0.5	2.0 LPCM	5 mM phosphate, 7.2	0.12 M NaCl	4 °C
0.5	2.0 LPCM	5 mM acetate, 4.5	0.12 M NaCl	4 °C
0.5	2.0 LPCM	5 mM acetate, 4.5	0.12 M NaCl	25 °C

LPCM = myristoyl lysophosphatidylcholine

mg/ml Glucagon	mM detergent	buffer, pH	Other additive	Temp- erature
1	2.5 HPS	5 mM phosphate, 7.2	0.2 M mannitol	4 °C
1	5.0 LPCL	5 mM phosphate, 7.0	0.12 M NaCl	4 °C
1	5.0 LPCL	5 mM phosphate, 6.0	0.12 M NaCl	4 °C
1	2.5 LPCL	5 mM phosphate, 7.2	0.2 M mannitol	4 °C
0.5	2.0 LPCL	5 mM phosphate, 7.2	0.2 M mannitol	4 °C
0.5	2.0 LPCL	5 mM phosphate, 7.2	0.12 M NaCl	4 °C
0.5	2.0 LPCL	5 mM acetate, 5.0	0.2 M mannitol	4 °C
0.5	2.0 LPCL	5 mM acetate, 5.0	0.2 M mannitol	25 °C
0.5	2.0 HPS	5 mM acetate, 4.5	0.12 M NaCl	25 °C

HPS = N-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate

LPCL = Lauroyl lysophosphatidylcholine

5 Example 5

Equilibrium solubility was determined as a function of pH for 2 mg/ml solutions of GLP-1(7-37) in the absence of additives and in the presence of increasing amounts of the detergent LPCL (lauroyl lysophosphatidyl choline). The results are shown in Fig. 5.

- 10 For solutions containing 2.5 and 5 mM LPCL, 100% solubility was observed throughout the entire pH range. 1.0 mM LPCL did not provide the full effect, although solubility was significantly enhanced relative to the reference composition without the detergent.

15 Example 6

Equilibrium solubility was determined as a function of pH for 2 mg/ml solutions of GLP-1(7-36)-NH₂ in the absence of additives and in the presence of increasing amounts of the detergent LPCM (myristoyl lysophosphatidyl choline). The results

- 20 shown in Fig. 6 illustrate that 2 mM LPCM provides full solubility in comparison with the reference composition.

Example 7

Equilibrium solubility was determined as a function of pH for 1 mg/ml solutions of the
5 acylated GLP-1(7-37) analogue N^ε-hexadecanoyl-γ-glutamyl-Lys26,Arg34-GLP-1(7-37)
in the absence of additives and in the presence of increasing amounts of the detergent
LPCL (lauroyl lysophosphatidyl choline). The results illustrated in Fig. 7 show that
1 mM and 2 mM LPCL enhance solubility relative to the reference composition, while
full solubility is obtained in the presence of 5 mM LPCL.

CLAIMS

1. An aqueous composition in the form of a solution comprising at least one peptide selected from glucagon, GLP-1, and analogues and derivatives thereof together with a stabilizing and solubilizing amount of at least one detergent, said detergent having at least 2 positive charges, at least 2 negative charges, or a combination of at least one positive charge and at least one negative charge, the peptide being present in a concentration of at least about 0.1 mg/ml, with the proviso that the detergent is not dodecyl phosphocholine.
2. A composition according to claim 1, wherein the peptide is present in a concentration of about 0.1-10 mg/ml, preferably about 0.2-5 mg/ml, e.g. about 0.5-3 mg/ml.
3. A composition according to claim 1 or 2, wherein the detergent is present in an amount of about 0.3-40 moles of detergent per mole of peptide, preferably about 0.5-30 moles of detergent per mole of peptide, more preferably about 1-20 moles of detergent per mole of peptide, such as about 1-10 moles of detergent per mole of peptide.
4. A composition according to any of claims 1-3, wherein the detergent comprises a polar head group and at least one aliphatic side chain, each aliphatic side chain having at least 6 carbon atoms.
5. A composition according to claim 4, wherein each aliphatic side chain has 8-20 carbon atoms, e.g. 10-18 carbon atoms.
6. A composition according to any of claims 1-5, wherein the detergent is a lysophospholipid, e.g. a 1-acyl-sn-glycero-3-phosphate ester of ethanolamine, choline, serine or threonine.
7. A composition according to claim 6, wherein the detergent is lauroyl or myristoyl lysophosphatidylcholine.

8. A composition according to claim 7 comprising about 0.5-2.5 mg of peptide per ml and about 1-3 mM of lauroyl or myristoyl lysophosphatidylcholine.
9. A composition according to any of claims 1-5, where the detergent is an N^α-
5 acylated derivative of lysine, arginine or histidine or a side-chain acylated derivative of lysine or arginine.
10. A composition according to any of claims 1-5, where the detergent is an N^α-
acylated derivative of a dipeptide, comprising a combination of a neutral amino acid
10 and lysine, arginine or histidine.
11. A composition according to any of claims 1-5, where the detergent is an N^α-
acylated derivative of a dipeptide X-X, X-Y, Y-X or Y-Y, where X is lysine, arginine or
histidine and Y is glutamic acid or aspartic acid.
- 15 12. A composition according to any of claims 1-5, where the detergent is an N^α-
acylated derivative of a tripeptide comprising a neutral amino acid and any
combination of two charged amino acids.
- 20 13. A composition according to claim 9, where the detergent is selected from the N^α-
decanoyl, N^α-lauroyl or N^α-myristoyl derivatives of L-lysine, L-arginine or L-histidine, or
from the corresponding side-chain acylated derivatives of L-lysine or L-arginine.
14. A composition according to any of the preceding claims, further comprising an
25 isotonic agent such as NaCl, glycerol or mannitol, a buffer substance such as acetate,
citrate or phosphate buffering at pH 4-8, and, optionally, a preservative such as
m-cresol, phenol or benzyl alcohol.
15. A method for stabilizing an aqueous composition in the form of a solution
30 comprising at least one peptide selected from glucagon, GLP-1 and analogues and
derivatives thereof, wherein the peptide is present in a concentration of at least about
0.1 mg/ml, the method comprising adding to said composition a stabilizing and
solubilizing amount of at least one detergent, said detergent having at least 2 positive
charges, at least 2 negative charges, or a combination of at least one positive charge

and at least one negative charge, with the proviso that the detergent is not dodecyl phosphocholine.

16. A method according to claim 15, wherein the aqueous composition is as defined
5 in any of claims 2-14.

17. A method for treating insulin dependent or non-insulin dependent diabetes mellitus or obesity in a patient in need of such treatment, comprising administering to the patient a therapeutically effective amount of an appropriate aqueous composition according to any
10 of claims 1-14.

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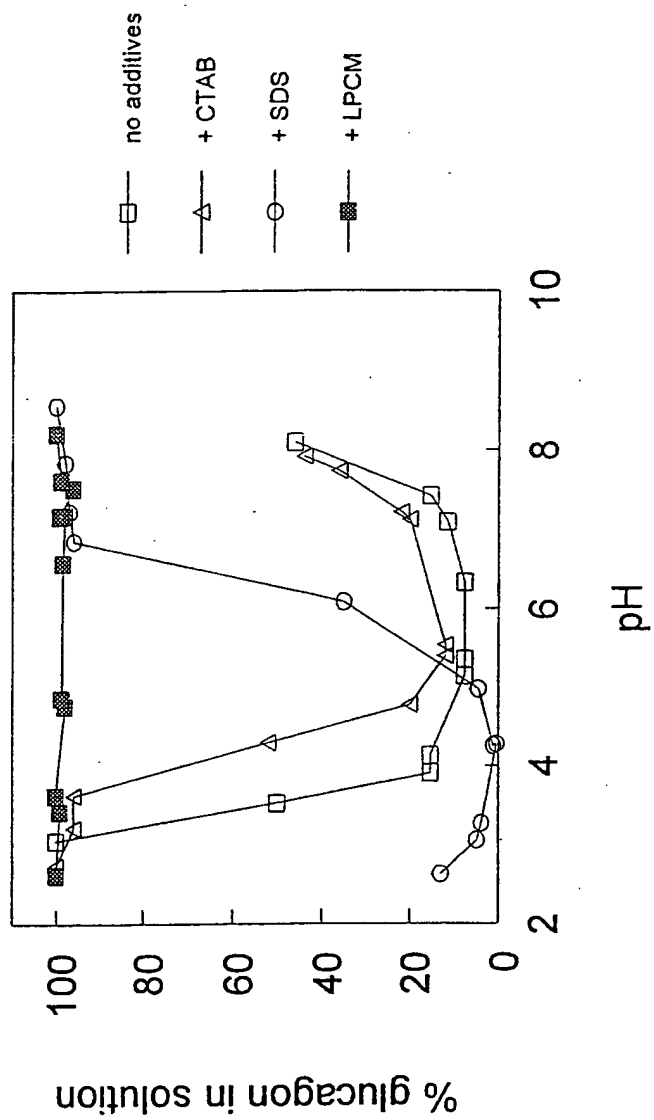


Fig. 1

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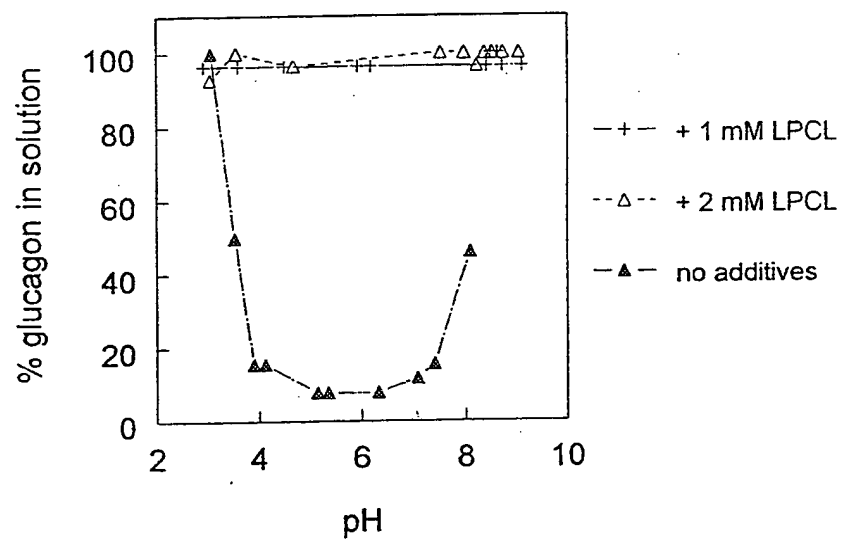


Fig. 2

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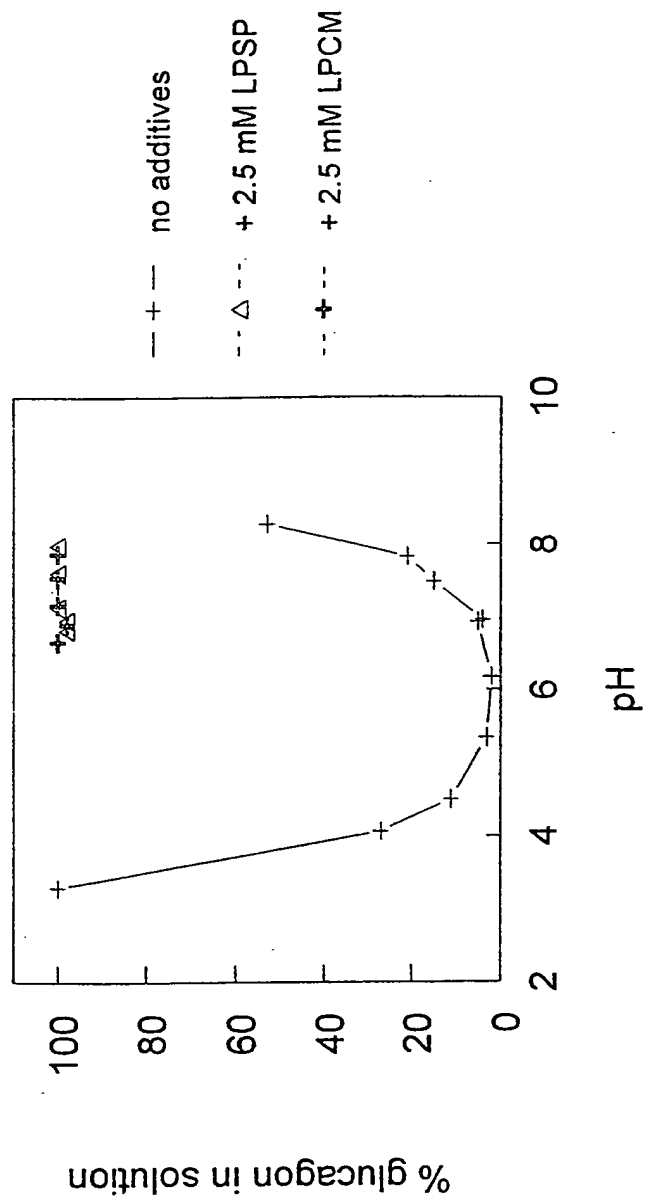


Fig. 3

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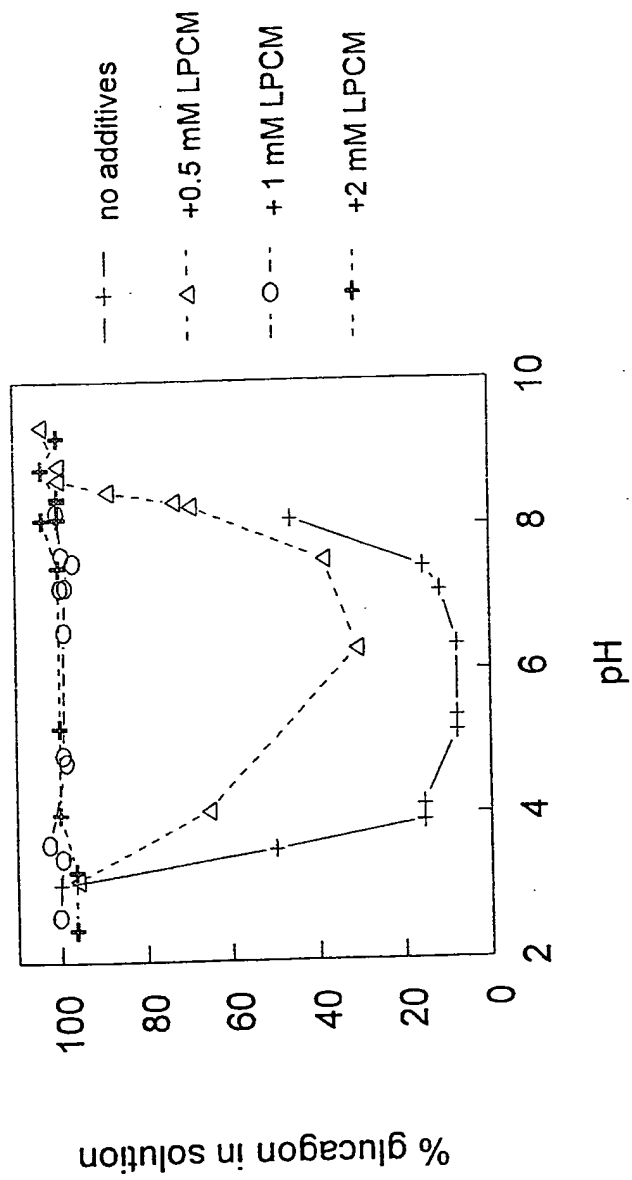


Fig. 4

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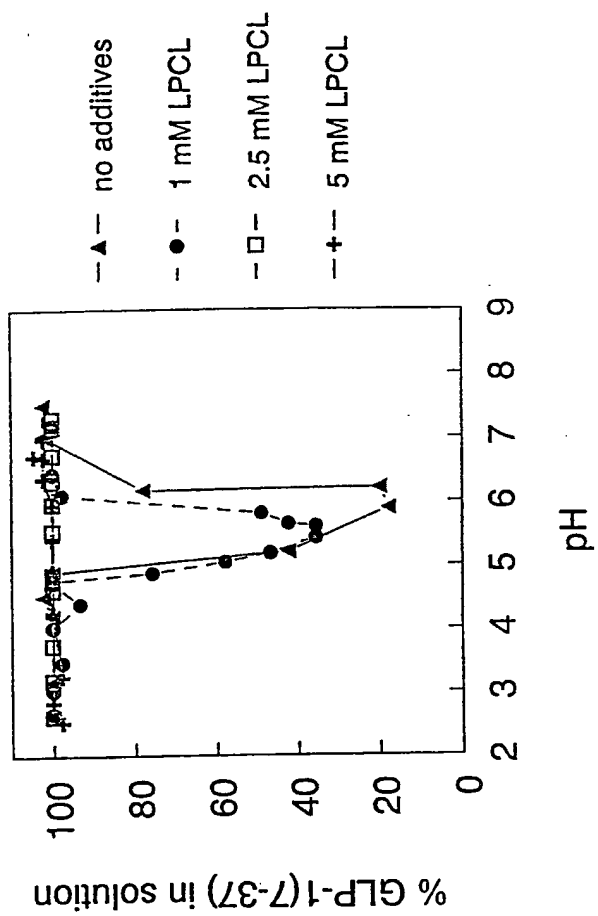


Fig. 5

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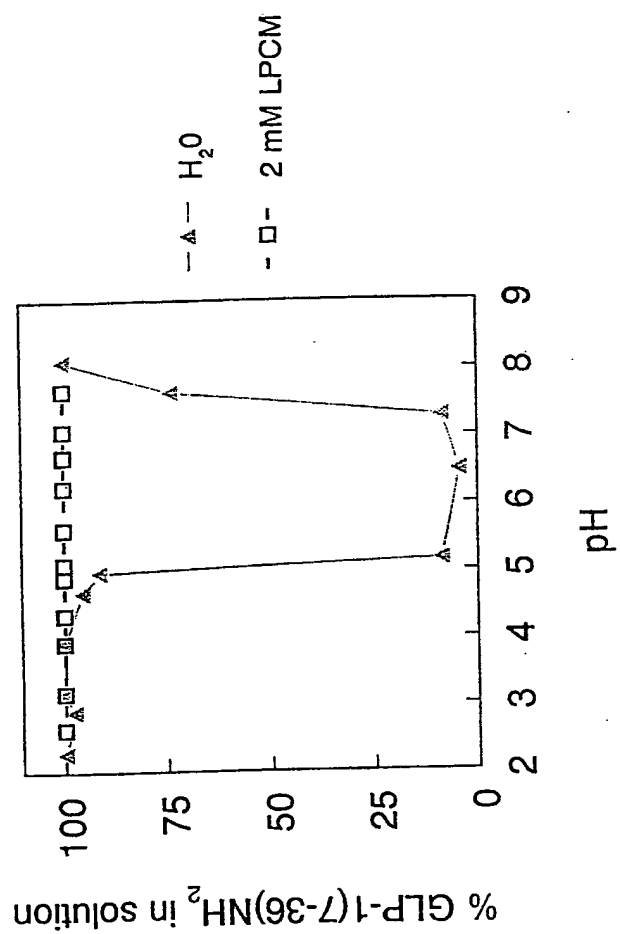


Fig. 6

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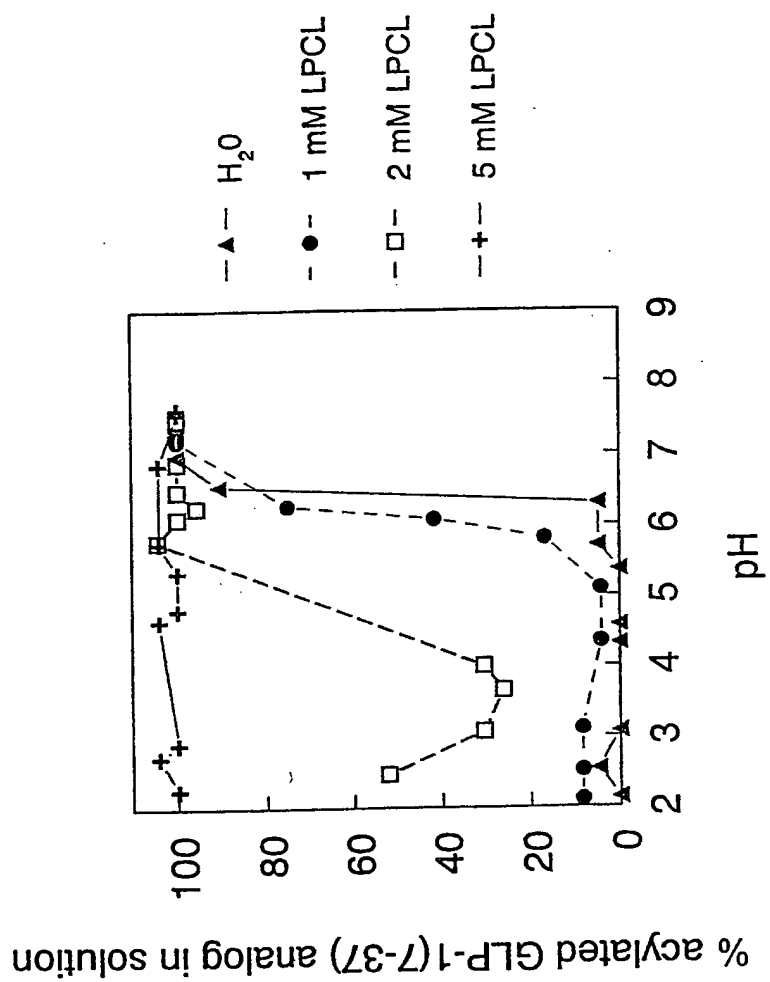


Fig. 7

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/DK 99/00115

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R.M. EPAND ET AL.: "MOLECULAR INTERACTIONS IN THE MODEL LIPOPROTEIN COMPLEX FORMED BETWEEN GLUCAGON AND DIMYRISTOYLGLYCEROPHOSPHOCHOLINE." BIOCHEMISTRY., vol. 16, no. 20, 1997, pages 4360-4368, XP002074758	1-5,15, 16
Y	EASTON, PA US see page 4360, right-hand column, line 9 - page 4361, left-hand column, line 4	14,17
Y	WO 95 32730 A (NOVO NORDISK A/S) 7 December 1995 see page 1, line 11 - line 12 see page 4, line 7 - line 25 --- -/-	14,17

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

8 June 1999

Date of mailing of the international search report

15/06/1999

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Fax: (+31-70) 340-3016

Authorized officer

Ryckebosch, A

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/DK 99/00115

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	C. BÖSCH ET AL.: "PHYSICOCHEMICAL CHARACTERIZATION OF GLUCAGON-CONTAINING LIPID MICELLES." BIOCHIMICA ET BIOPHYSICA ACTA, vol. 603, no. 2, 12 December 1980, pages 298-312, XP002074759 AMSTERDAM, NL cited in the application see page 305, line 6 - line 28 ---	1-17
A	GB 1 202 607 A (NOVO TERAPEUTISK LABORATORIUM A/S) 19 August 1970 cited in the application see the whole document -----	1-17

INTERNATIONAL SEARCH REPORT

Inter. ...onal application No.

PCT/DK 99/ 00115

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 17
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 17
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/DK 99/00115

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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